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Serial Measurement of the Cortical Blood Flow in the Canine Renal Allografts During the Rejection Crisis

by

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INTRODUCTION

Up to the present, numerous renal homotransplantations have been practiced on human beings and animals with various methods. But, nowadays, a difficult problem still remains for us to be solved. It is not a matter of the refinement of operating technique but the subjugation of the immunologic reaction of the recipient against the transplanted kidney. Since the ideal immunosuppressive method without any toxicity has not been established, adequate control of the reaction for the renal allograft can, so far, be achieved with the precise manifestation of this immunologic reaction and the correct clinical judgement in the use of immunosuppressive drugs. And it is most important to detect the rejection crisis as early as possible ; however, it has been suggested that the usual parameters of renal function were inadequate for this measure especially in modified recipients. A useful method to monitor the effect of the immunosuppressive drug therapy also has not been demonstrated.

The evidences obtained from several investigations indicate that ischemia may act an important role in rejection ; and some of the evidences obtained with arteriogram[9)10)] and measurement of intra-renal distribution of blood flow[48)] suggest that ischemia is more prominent in the cortical tissue than in other parts of the kidney allograft. It was also reported that the histological changes in allografted dog kidney started in the cortex and were always more advanced there[10)8)]. In spite of these considerable evidences suggesting the cortical ischemia in renal allograft, any report of direct measurement on the hemodynamic changes occuring in the cortical tissue has not appeared.

The measurement of blood flow by means of the thermo-electric principle was devised by REIN[36)]. This method has been evolved and utilized by many workers[40)2)16)42)14)15)]. In the experiment herein described, it was decided to adopt the double thermocouple introduced by KIESE, in 1957[25)], and continuous blood flow measurement was made with the double thermocouple method reported by TAKAHASHI[46)], and TSUNEKAWA et al.[49)] With the double thermocouple embedded in the canine renal cortical tissue, the changes in the cortical blood flow induced by the transplantation and the rejection was directly and serially defined ; the effects of prednisolone and acetylcholine on the cortical blood flow was studied in the renal allografts undergoing rejection ; and the response of cortical circulation to nor-epinephrine was studied on the transplanted, i. e. completely denervated

kidneys.

MATERIALS AND METHODS

Materials

These experiments were performed using mongrel dogs of unknown genetic history ranging in weight from 7 to 13 kg.

Renal transplantation

Kidney grafts were transplanted into the thigh of the dog for the similar reason of HUME[20], namely, that was less traumatic and it gave less unwanted influence on the recipient to place the kidney graft in the thigh rather than in the iliac fossa. The renal vessels were anastomosed end to end with the femoral vessels by running sutures with 7-0 nylon. Usually the left kidney was transplanted to the right thigh and vice versa. The anoxia time for transplanted kidneys was generally from 20 to 30 minutes. The ureter was brought out through the small incision in the skin for the convenience of observation on the functional state of the graft. The kidney was buried in a pocket created between the subcutaneous tissue and the muscles in the medial aspect of the thigh.

Measurement of cortical blood flow

The double thermocouple is formed of two copper-constantan thermocouples which join together at one point. The copper and the constantan wires are 0.1 mm in diameter. Electric insulation is obtained by covering the wires with enamel and vinyl paint. One thermocouple included in the heating circuit is effected by direct electric current and heats the hot junction of the other thermocouple included in the measuring circuit. Direct electric current is supplied by a 6 volt storage battery in series with a suitable ammeter and rheostats (Fig. 1).

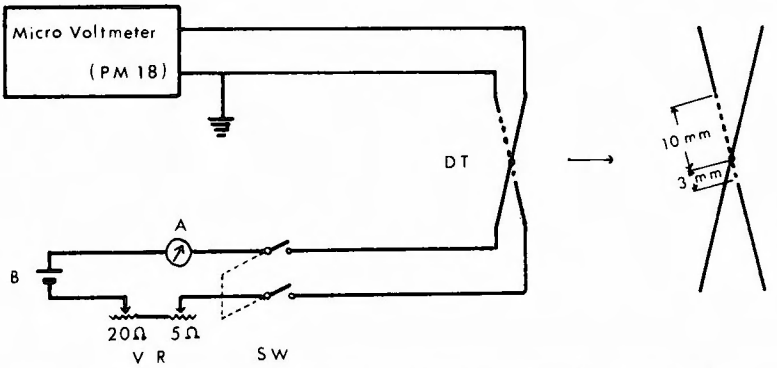

Fig. 1 Wiring diagram of double thermocouple method. Insert shows large scale drawing of double thermocouple. Solid line, copper wire ; broken line, constantan wire ; A, milliammeter ; B, battery ; DT, double thermocouple ; VR, variable resistance ; SW, switch.

By the difference of temperature between the hot junction and the cold junction of the measuring thermocouple, thermoelectric current is generated in the measuring circuit and is measured with a microvoltmeter of chopper type (Model PM 18, Toa Denpa Kogyo Co. Ltd.). It has been shown that the square of the amperage of heating circuit is a

linear function of blood flow, if the voltage generated in the measuring circuit is kept constant[15]. In the present experiments, the voltage was accurately maintained at 100 mV by current adjustment with the rheostats in the heating circuit. The cooling power of a stream depends upon its velocity, therefore, when the blood flow increment (or decrement) occurs, the heating current must be increased (or decreased) in order to maintain the temperature constant at the hot junction in the measuring thermocouple. So, the blood flow can be measured by the ammeter in the heating circuit.

The continuous blood flow measurement was made by the method reported by TAKAHASHI[46], and TSUNEKAWA and his coworkers[49] using double thermocouple and recorded on a polygraph (Unicorder, Model UR 225, Nippon Denshi Kogaku Co. Ltd.).

After the transplantation of a kidney, a double thermocouple was introduced into the cortex of the kidney graft by a straight needle. The hot junction and the cold junction of the measuring thermocouple were embedded in parenchyma of renal cortex at the depth of approximately 5 mm from the surface of the kidney. Four leading wires from the double thermocouple were led to the exterior through the subcutaneous tissue and a stab wound in the flank, outside of the thigh, or the back of the dog, and sheltered from biting and scratching by the dog with an aluminum cylinder of 10 mm in depth, 50 mm in diameter with a cap (Fig. 2).

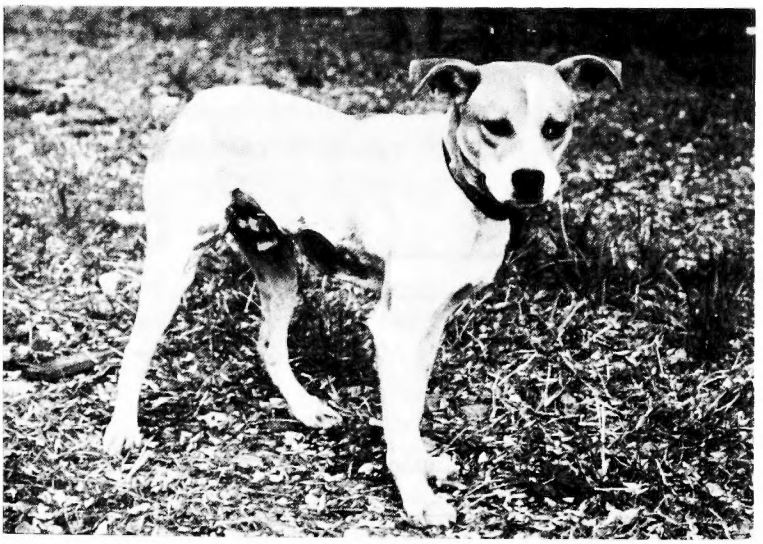

Fig. 2 View of a dog's flank, showing aluminum cap covering lead wires. (Series 3, No. 5)

The initial measurement of renal cortical blood flow was made at 30 minutes after the performance of anastomosis of the vessels and the square of the heating current at this time was stipulated as 100 % of the blood flow in this kidney. At the same time, the zero blood flow was established by mechanical occlusion of the renal artery, and the square of the heating current on this occasion was kept to be 0 % of the blood flow of the kidney. Then the measurement of the cortical blood flow was made daily following the initial measurement and put on record in percentaged form.

Blood pressure measurement

The continuous measurement of mean blood pressure in the contralateral femoral artery opposite the artery used for transplantation was made using a electronic manometer (Model MP 4T, Nihon Koden Co. Ltd.) and was recorded on a multipurpose polygraph (Model RM 150, Nihon Koden Co. Ltd.).

Renal angiography

Urographine 20ml was usually utilized as the radiographic contrast agent. For the delivery of the contrast agent, a polyethylene catheter, approximately of 2mm outside and 1.5 mm inside diameter, was introduced into the lower part of the aorta via the femoral artery.

Measurement of kidney size

The size of the kidney graft was monitored with two silver CUSHING- clips which were applied to each pole of the kidney graft to act as X-ray markers at the time of the kidney transplantation.

Histological method

All kidneys were fixed in 10 % formalin. The stain used was hematoxylin and eosin.

Immunosuppressive method

Immunosuppressive drug therapy with azathioprine and prednisolone was used. Azathioprine, 25-50 mg/day, was given orally for 1 to 4 days prior to the homotransplantation and continued as long as the experiment required. When the significant fall in the cortical blood flow occurred, 40 mg of prednisolone was injected intramuscularly or intravenously.

EXPERIMENTAL GROUPS

First series : Normal kidney

For the purpose of approving the stability of this measuring system, and examining the influence of the double thermocouple upon the kidney, the measurement of the cortical blood flow was serially made on normal kidneys.

Second series : Autograft

(a) In order to examine the influence of the operation upon the cortical blood flow, the serial measurement of blood flow was made on autografts.

(b) To study the influence of the denervation caused by transplantation on cortical blood flow, the continuous measurement of blood flow was made on the autografts using nor-epinephrine. The continuous measurement of blood pressure in the femoral artery was made simultaneously.

Third series : Unmodified allograft

(a) In order to define the changes in cortical blood flow induced by the rejection, the blood flow was measured serially on unmodified renal allograft. To compare with the changes in cortical blood flow in the allograft, the radiographic measurement of the size of the kidney graft was made daily, and the nephro-arteriogram was taken on the 2nd, 4th, and 6 th postoperative day.

(b) In an attempt at studying the effect of prednisolone, nor-epinephrine and acetylcholine

on cortical blood flow, the continuous measurement of blood flow was made on unmodified allografts. The continuous measurement of blood pressure was made simultaneously.

Fourth series : Modified allograft

Monitoring the effect of immunosuppressive drugs, the cortical blood flow was measured serially on modified allografts.

RESULTS

First series

The behavior of cortical blood flow in 6 normal kidneys was observed. In 3 cases, the leading wires were broken down by the action of the dog by the 5 th experimental day. In 3 successful experiments, the renal cortical blood flow showed no distinguishable change in their determination during 1 to 2 weeks (Fig. 3). At the end of these examinations, the kidneys were removed and microscopically examined. No remarkable damage with the double thermocouple against the cortical tissue was observed in the area where it had been inserted (Fig. 4).

Second series

(a) The cortical blood flow was measured daily on 3 autografts. In 2 cases, the cortical blood flow was maintained at virtually equal level in the autografts similarly to that in the normal kidney. In an exceptional case in which a considerable amount of bleeding had discharged in the course of the operation, there was a progressive increase in the cortical blood flow until the 2nd postoperative day. Thereafter, no significant change was noted in the cortical blood flow.

(b) The influence of denervation on cortical blood flow was examined on 5 auto-

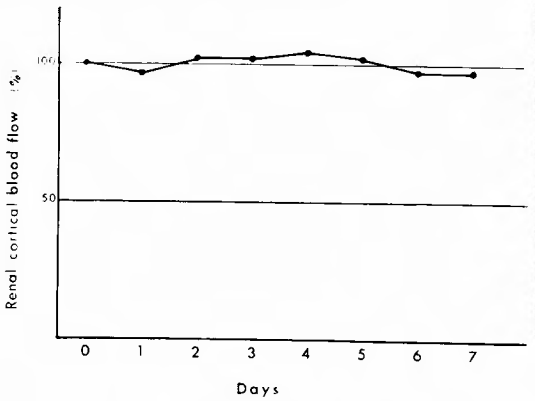

Fig. 3 Daily cortical blood flow in the normal kidney. (Series 1, No. 2)

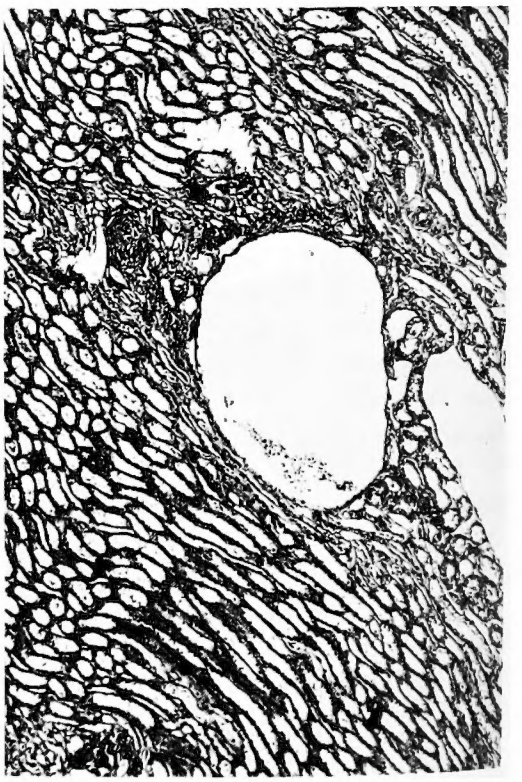

Fig. 4 Section from a normal kidney removed on the 14th day of serial measurement where double thermocouple was embedded in. Little amount of hemorrhage can be seen. Hematoxylin-eosin. ×40. (Series 1, No. 3)

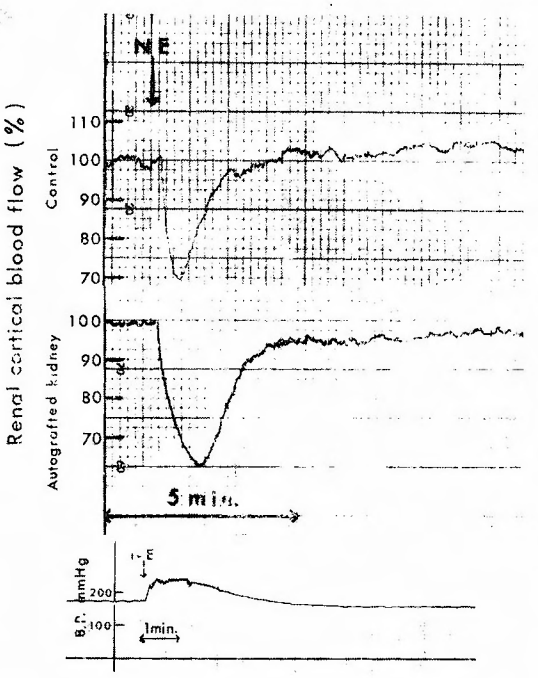

Fig. 5 The effect of nor-epinephrine on cortical blood flow in the normal and autografted kidneys on the 3rd postoperative day. Simultaneous blood pressure measurement is illustrated, too. NE, 0.1mg of nor-epinephrine ; BP, blood pressure. (Series 2, No. 4)

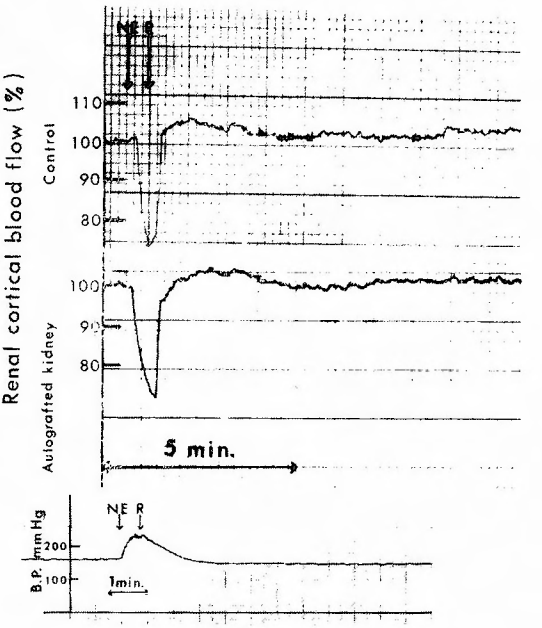

Fig. 6 The effect of nor-epinephrine and Regitine on cortical blood flow in the normal and autografted kidneys on the 3rd postoperative day. Simultaneous blood pressure measurement is also illustrated. NE, 0.1mg of nor-epinephrine ; R, 1mg of Regitine ; BP, blood pressure. (Series 2, No. 4)

grafted kidneys using nor-epinephrine. As the control, the response of cortical blood flow in the normal kidney of the same dog was examined simultaneously. A dosage of 0.1 mg of nor-epinephrine was given intravenously to the dog bearing the hemilaterally autografted kidney on the 3 rd day after the transplantation. There was an initial sharp and rapid fall followed by a sharp rise in the blood flow in both normal and autografted kidneys' cortices (Fig. 5). The reduction of cortical blood flow was from 20 to 35 % below the initial level ; and it seemed that the degree of the reduction in blood flow varied with the measure of blood pressure of the dog. The reduction in the cortical blood flow became more significant accordingly as the blood pressure of the dog rose. The reduction in cortical blood flow induced by nor-epinephrine lasted approximately 2.5 minutes in the autografted kidney, while 1.5 minutes in the control kidney. This action of nor-epinephrine was immediately and entirely blocked by the subsequent intravenous administration of 1 mg of Regitine (Fig. 6). On the 10 th postoperative day, the reduction of cortical blood flow caused by nor-epinephrine lasted about 3 minutes ; and the response curve was transformed into two peaks in shape (Fig. 7). On the 30 th postoperative day, no further changes were noted in the cortical blood flow response to nor-epinephrine.

Third series

(a) The hemilateral homotransplantations were performed on 11 dogs of which own kidneys were left intact.

The serial measurement of cortical blood flow was made on 8 unmodified allografts. In an exceptional case, the cortical blood flow fell suddenly to 0 %, and allograft became anuric on the next operative day. The graft was removed and the renal artery was found to be clotted on the 3rd day after the transplantation. The remaining cases were considered to be satisfactory.

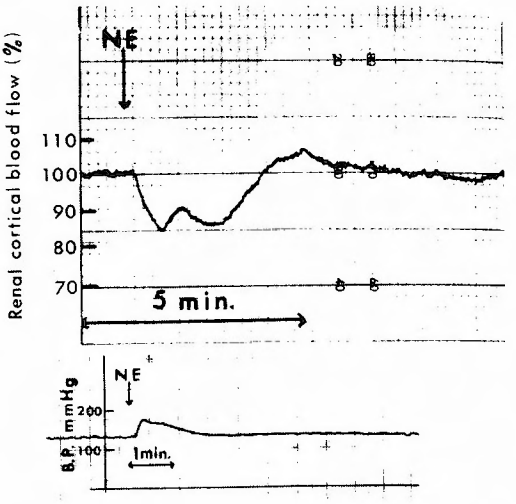

Fig. 7 The effect of nor-epinephrine on cortical blood flow in the same autografted kidney in Figs. 5 and 6 on the 10th day after the transplantation. Simultaneous blood pressure measurement is also illustrated. NE, 0.1mg of nor-epinephrine ; BP, blood pressure. (Series 2, No. 4)

In 4 cases, the obvious decline in the cortical blood flow occurred on the 3 rd or 4 th postoperative day, and in one case, on the 5 th postoperative day. During 2 to 3 days thereafter, the cortical blood flow decreased progressively and drew toward 0 % (Fig. 8). One day after the sudden decrement of blood flow, swelling of the graft was manifested with the radiological method (Fig. 9). One or two days still later, the kidney became anuric, the attenuation of renal vessels was apparently observed on the angiography (Figs. 10 and 11), and the late phase of the rejection including disintegration of glomerular and tubular architecture was noted

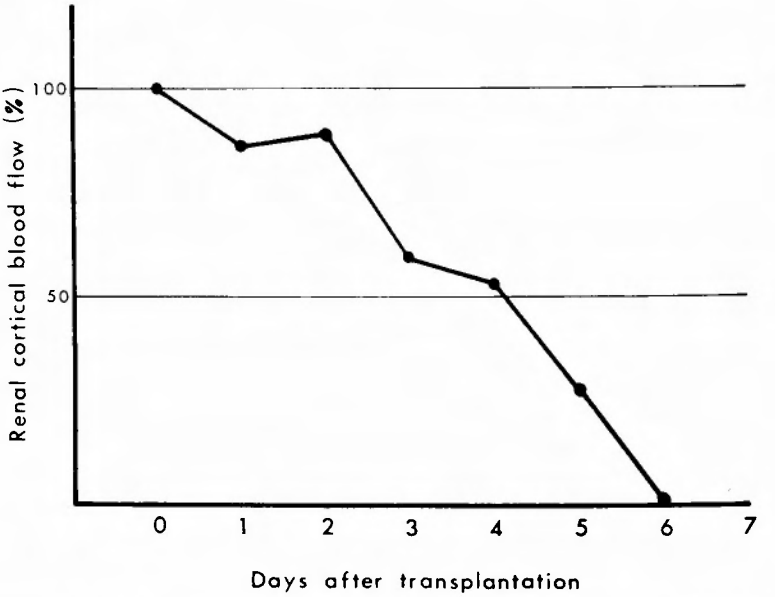

Fig. 8 Serial change in the renal cortical blood flow in unmodified allograft on the days following transplantation. (Series 3, No. 1)

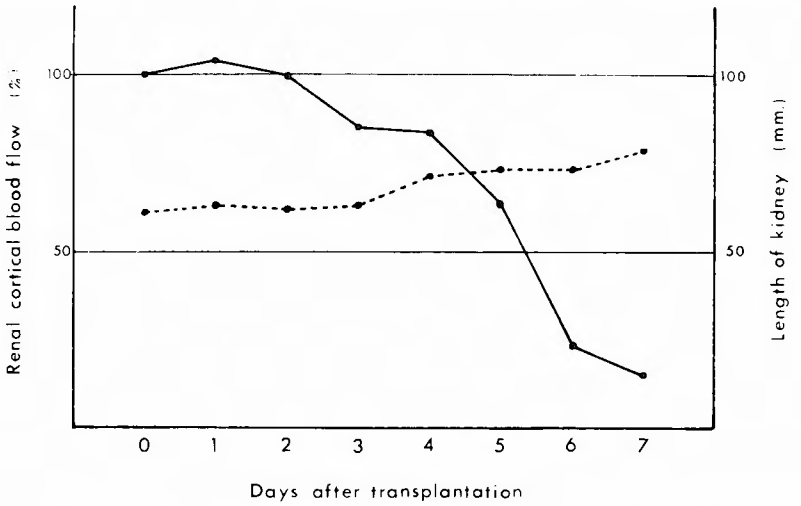

Fig. 9 The relationship between the change in renal cortical blood flow and the development of swelling of kidney allograft. Solid line, renal cortical blood flow ; broken line, length of kidney. (Series 3, No. 5)

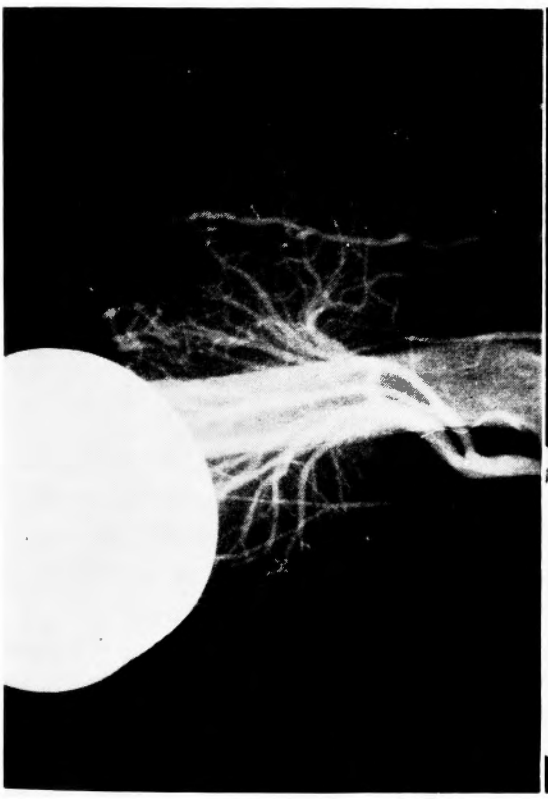

Fig. 10 Photograph of a nephro-arteriogram of the same kidney depicted in Fig. 8, functioning well on the 3rd of postoperative day. A white large round shadow is an aluminum cover of lead wires. (Series 3, No. 1)

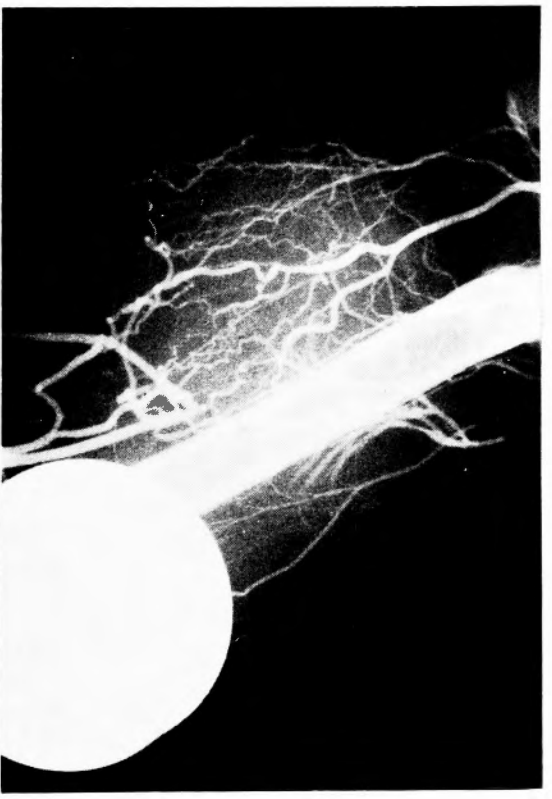

Fig. 11 Photograph of a nephro-arteriogram of the same kidney depicted in Figs. 8 and 10, which stopped secreting urine on the 6th postoperative day. (Series 3, No. 1)

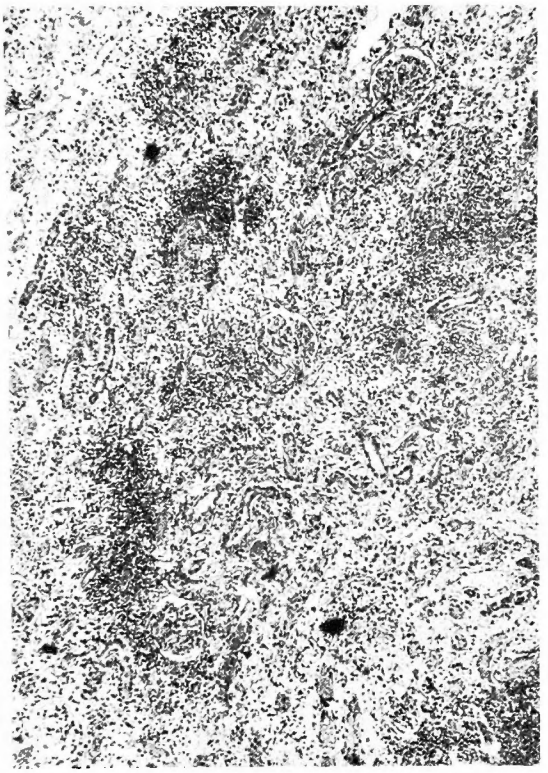

Fig. 12 Section of the transplant from the experiment depicted in Figs. 8, 10, and 11, which was removed on the 6th day after the transplantation, shows heavily infiltrated interstitial tissue with mononuclear cells, and wide spread degeneration of tubules. Hematoxylin-eosin, ×40. (Series 3, No. 1)

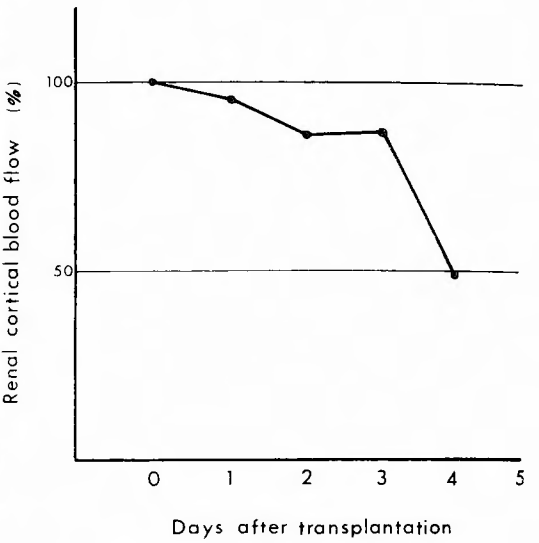

Fig. 13 Serial change in the cortical blood flow in unmodified allograft on the days following transplantation until the removal on the 4th postoperative day. (Series 3, No. 6)

by the histological examination (Fig. 12).

In 2 cases, the renal cortical blood flow declined to the level below 50 % on the 4 th postoperative day (Fig. 13) ; and the transplants were removed and histologically examined. In these cases, it was found that the mononuclear cell infiltration had evidently occurred but the glomerular and tubular architecture was still preserved essentially normal in the renal cortex, while the angiography showed a respectable amount of blood flow to be kept in the kidney allografts (Figs. 14 and 15).

(b) The continuous measurement of cortical blood flow and blood pressure in the femoral artery was designed on 2 unmodified allografts to elucidate the effect of prednisolone on the cortical blood flow just after the injection of it. On the day of the transplantation, the continuous measurement of cortical blood flow in the renal allograft showed no remarkable change for 3 hours following the intravenous injection of prednisolone 40 mg (Fig. 16). On the 3 rd postoperative day when the cortical blood flow decreased to approximately 70 %, a very slow increase in blood flow was observed starting about 15 minutes after the injection of prednisolone. The gradual increase in the cortical blood flow continued during 3 hour period of observation, while the blood pressure in the

Fig. 14 Section of the kidney in the same animal as in Fig. 13, which was removed on the 4th postoperative day, shows obvious mononuclear cell infiltration in the interstitial spaces. Hematoxylin-eosin, ×40. (Series 3, No. 6)

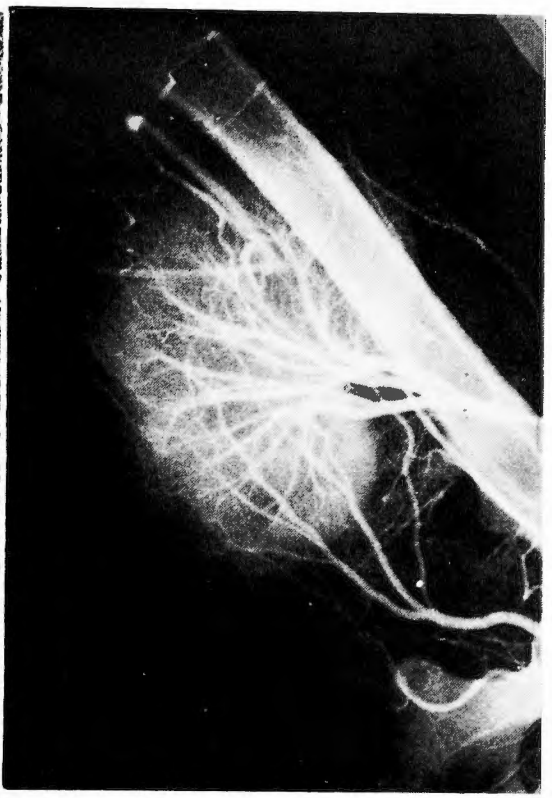

Fig. 15 Photograph of a nephro-arteriogram of the same kidney depicted in Figs. 13 and 14, on the 4th postoperative day. (Series 3, No. 6)

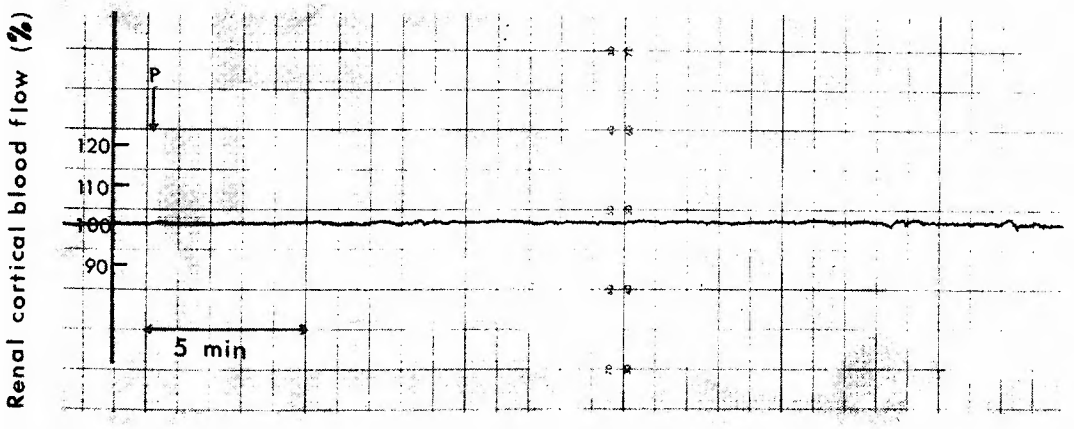

Fig. 16 The effect of prednisolone on the cortical blood flow in unmodified allograft on the day of transplantation. No remarkable change can be seen in the renal cortical blood flow. (Series 3, No. 9)

femoral artery showed no remarkable alteration during the measurement (Fig. 17).

The response of cortical blood flow to nor-epinephrine and acetylcholine was examined

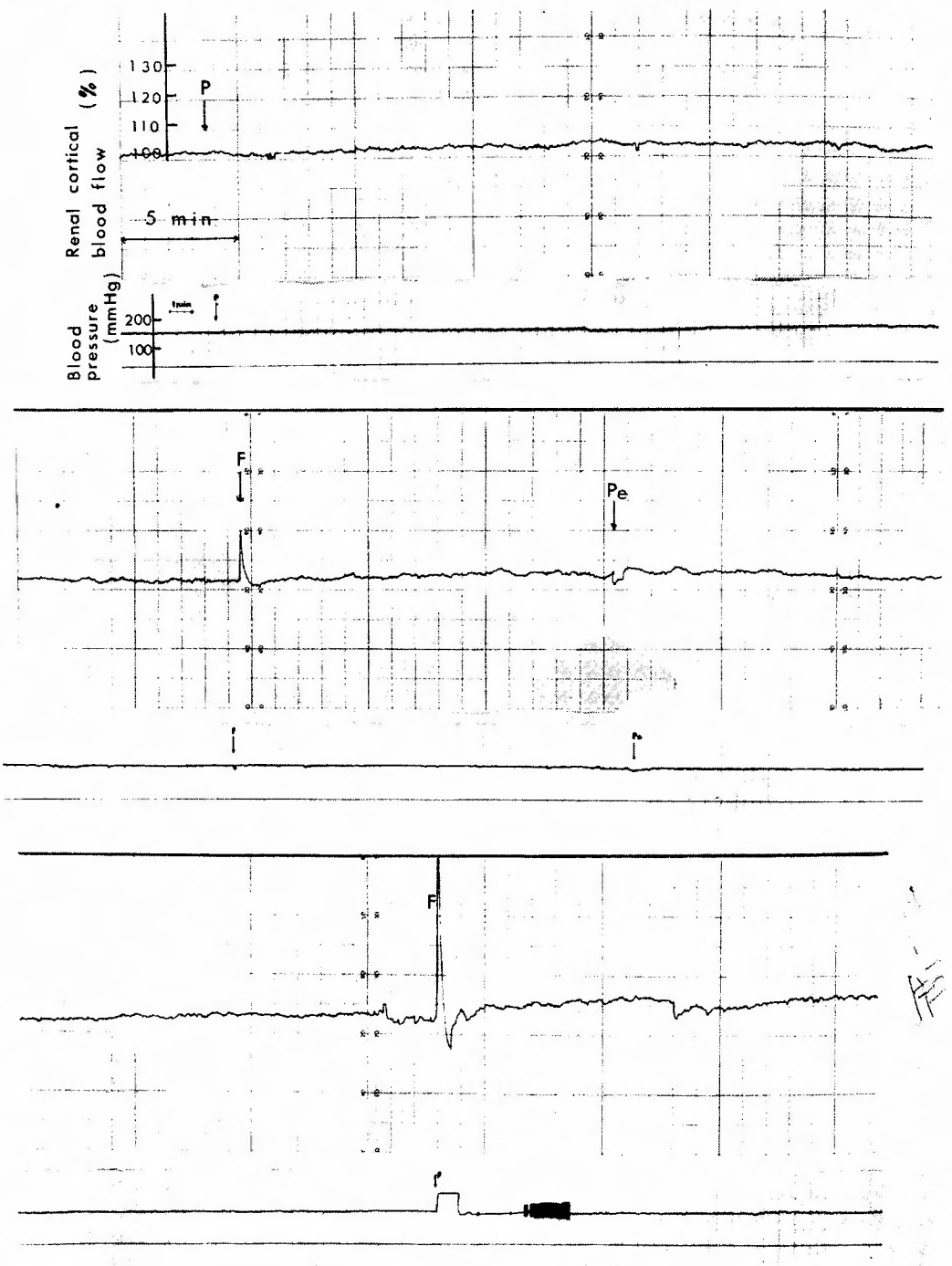

Fig. 17 The effect of prednisolone on the cortical blood flow in unmodified allograft on the 3rd postoperative day. A gradual increase in cortical blood flow can be seen. Simultaneous measurement on blood pressure shows no remarkable change. P, 40mg of prednisolone ; Pe, 10mg of pentobarbital sodium ; F, heparinized saline flush into the catheter of electronic manometer which is inserted into the femoral artery. (Series 3, No. 9)

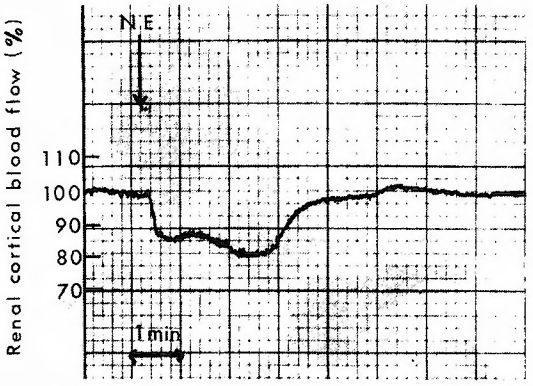

Fig. 18 The response of cortical blood flow in allografted kidney to nor-epinephrine on the 6th day after the transplantation. NE. 0.1mg of nor-epinephrine. (Series 3, No. 9)

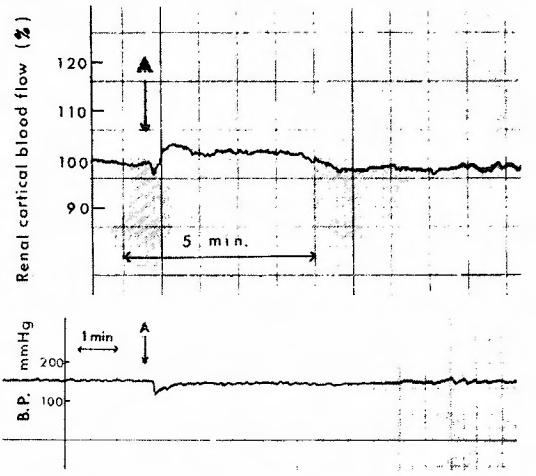

Fig. 19 The response of cortical blood flow in allografted kidney to acetylcholine on the 4th postoperative day. Simultaneous blood pressure measurement is illustrated, too. A, 0.01mg of acetylcholine ; BP, blood pressure. (Series 3, No. 10)

with the continuous measurement of blood flow on 3 unmodified allografted kidneys. A dosage of 0.1 mg of nor-epinephrine was given intravenously to the dog on the 6 th day after the transplantation. The response of cortical blood flow to nor-epinephrine in allografted kidney was quite similar to that in the autografted kidney on the 10th day (Fig. 18).

A dosage of 0.01 mg of acetylcholine was administered intravenously to 3 dogs bearing allografted kidney on the 4th postoperative day. One out of 3 cases showed slight increase in the continuous measurement of the cortical blood flow ; and 3 minutes later, the blood flow returned to the initial level, while in other cases, no remarkable change resulted from the injection of acetylcholine (Fig. 19).

Fourth series

The serial measurement was made on the cortical blood flow in the allografted kidneys modified with azathioprine. Allografts were bilaterally transplanted to both thighs in each of 7 recipients in which own kidneys were removed. One recipient died of anesthesia postoperatively. In 4

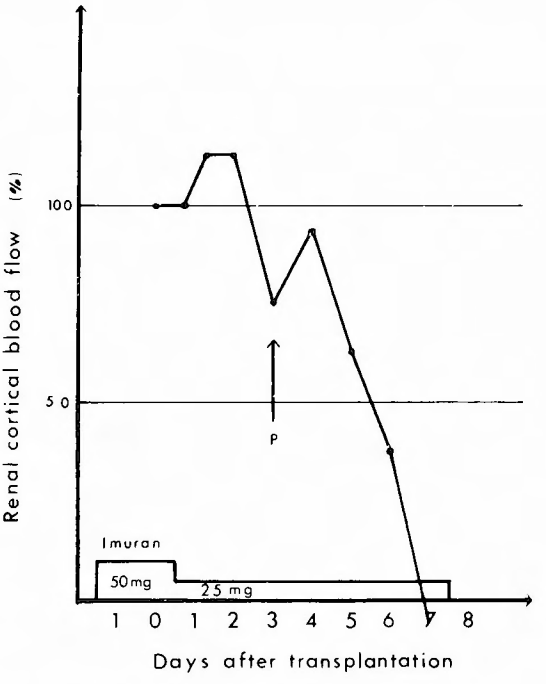

Fig. 20 Typical experiment in the animal treated with azathioprine which rejected transplant. Arrow indicates the injection of prednisolone 40mg. (Series 4, No. 2)

cases the serial measurement of cortical blood flow in the hemilateral kidney grafts was carried out for 7 to 15 days. In the remaining kidneys, other troubles occurred in the leading wires by the 5 th postoperative day. In the 4 successful cases the cortical blood flow was kept virtually constant by medication of azathioprine 50 mg/day ; and a decrease in cortical blood flow occurred when the dosage was reduced to 25 mg/day. During the process of the decrementation of cortical blood flow in these cases, 40 mg of prednisolone was injected. Partial recovery of blood flow was observed on the next day after the injection (Fig. 20). In one case the measurement of cortical blood flow was made 4 hours after the injection of prednisolone. It was found that partial recovery of the blood flow had already occurred. Twelve hours after the injection the blood flow was kept to be the same level as that of 4 hours, and additional administration of prednisolone 40 mg at this time brought about no further recovery of the blood flow thereafter (Fig. 21).

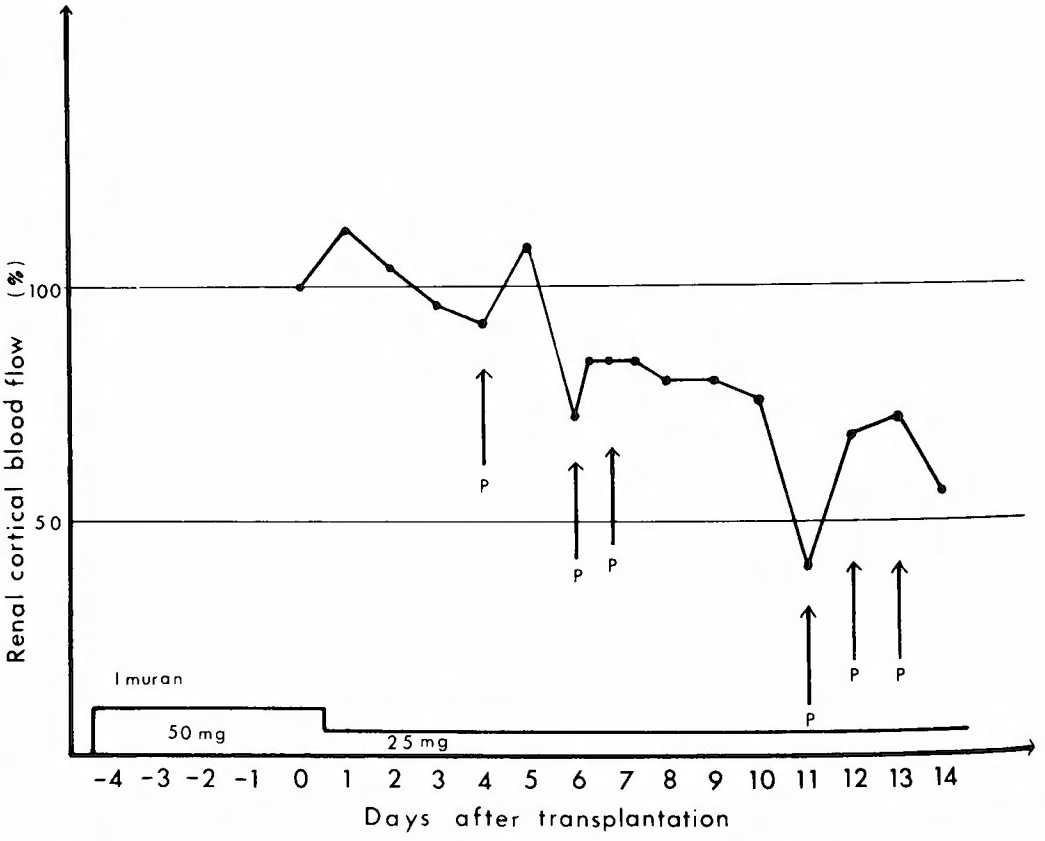

Fig. 21 Typical experiment in the animal treated with azathioprine and prednisolone. On the 6th postoperative day, recovery in the cortical blood flow resulted with prednisolone can be seen 4 hours after the administration. It is shown that the cortical blood flow is kept to be the same level during 24 hour period in spite of the additional injection of prednisolone. Arrow indicates the injection of prednisolone 40mg. (Series 4, No. 5)

DISCUSSION

The evidence which is suggesting renal ischemia in the allograft has been obtained with histologic studies[43)20)19)34)35)], histochemical and enzymatic investigations[22)53)], electron microscopic investigation[28)], and arteriographical studies[9)10)26)].

A decline in the renal blood flow was proved by ^{131}I Hippuran clearance in the allograft undergoing rejection which reduced the renal function[29)]. If the renal tubular impairment does not occur under the complete renal ischemia during the transplanting period, the cortical ischemia in the allograft undergoing rejection might be implied from the result obtained with this method. However, not only the continuous measurement of blood flow can not be made with this method, but also this method is too expensive to be adopted in the frequent measurements because the method is obliged to employ isotope and scintillation counter.

Investigations were also undertaken to define the hemodynamics of renal allografts with direct measurement of the total blood flow in the kidney by some workers. For the measurement of blood flow in animals, several methods have been evolved since early times[47)6)44)24)27)23)13)]. DEMPSTER cut the renal vein and measured the blood outflow[10)11)]. KOUNTZ et al.[28)] and WILLIAMS et al.[55)] determined the renal venous flow of the canine allografts using a constant local indicator dilution method[41)]. WILLIAMS explained that their method was not ideal because of the high mortality.

The measurement of arterial blood flow in renal allografts was also made by some investigators using a electromagnetic flow meter. The measurement on the renal allografts by this method, however, have been limited mainly to single observation because of its technical difficulties. In order to understand the hemodynamics in the transplant completely, it is neccessary to elucidate how the blood flow varies with time. Only JACKSON et al.[21)] made a serial determination on blood flow in renal allografts undergoing rejection with electromagnetic flow meter using a probe implanted into the dog. They described arterial obstructions caused by their device occuring in some instances. Then, RETIK et al.[37)] determined the renal blood flow with the electromagnetic flow meter on the day of transplantation, and repeated their efforts serially at laparotomy generally at 2 to 3 day intervals.

Notwithstanding these considerable efforts, these total blood flow measurements do not reflect the cortical hemodynamic changes in the renal allografts which has been emphasized by many other workers in this problem because of the redistribution of intrarenal blood flow induced by the immunologic reaction[48)38)39)]. For these reasons, another method had to be sought in the present experiment for the sake of the direct and continuous measurement of the blood flow in the renal cortical tissue.

The measurement of the blood flow by means of the thermo-electric principle was originally devised by REIN[36)]. GIBBS[14)] developed a thermo-electric blood flow meter in the form of a needle which was found by many workers to be useful for the measurement of vascular changes in internal organs. GRAYSON et al.[15)] made appreciable progress in the physical basis of the application of thermo-electric principle using a modified model of needle flow meter with an improved circuit. By now, this method has been evolved still more and utilized in the blood flow measurement in the parenchymal organs

by many investigators. So far as the author is aware, there are, however, no published reports of the serial experiments using a thermo-electric blood flow measurement.

From experimenting, it was known to be rather difficult to succeed in obtaining the serial results using this method. The thermocouples and leading wires were sometimes broken down within the experimental period. Of 31 cases of serial measurements, troubles occurred on the wires in 11 cases. None the less, in the serial measurements of cortical blood flow in the first series, the action of the measuring system of this method proved to be valid and stable. It was also confirmed that the measurement of blood flow with this method gave no dangerous influences on the whole body and the kidney of the animal.

As the result from the second series, it was confirmed that the cortical circulatory system in the transplanted kidney acquires hypersensitivity to nor-epinephrine. This hypersensitivity resulted in a 70% increase in the blood flow response to nor-epinephrine in the autograft over the normal kidney on the 3rd postoperative day, and a 100% increase on the 10th postoperative day. The transplanted, meaning completely denervated kidneys initially produce a large volume of urine with a low specific gravity. In dogs, the autotransplants will continue to produce urine, increasing the concentration, and reaching normal value after 2 to 3 weeks[9]. It is considered that the transplanted kidney loses control of nervous system, gradually acquires hypersensitivity to vasoconstrictive agents, and comes to be under the control of humoral system during several days after the transplantation. On the other hand, in the present experiments, it was observed that a response curve of cortical blood flow to nor-epinephrine was transformed into two peaks in shape by the 10 th postoperative day in both auto- and allografts. Although the meaning of transformation of the response curve of cortical blood flow remains obscure, it might be considered that this phenomenon suggested the functional change of the α-receptor to nor-epinephrine in the vascular system of renal transplant, or the existence of plural components in the α-receptor of renal cortex.

From the results of the third series, it was ascertained that the method is a means of value in detecting an early rejection crisis. The serial measurement of blood flow detects the rejection crisis in the canine renal allografts 1 to 3 days earlier than other indices, such as a decline in total renal blood flow, a decrease in urine output, and the swelling of the graft. It is clarified that when the incipient decline in cortical blood flow is observed, the mononuclear cells evidently infiltrate, but the architecture is still preserved to be essentially normal in the cortical tissue in this period ; and the reduced cortical blood flow in the renal allograft can be partially recovered with the administration of prednisolone.

In the fourth series of experiments, the method proved to have a considerable value in the serial study on monitoring the effect of immunosuppressive drugs on rejection. It was found that the partial recovery of cortial blood flow was brought about by prednisolone and completed within 4 hours following the administration of this drug in the renal allograft undergoing rejection.

Some investigators who made the measurements on total blood flow in the canine renal allografts, as already mentioned, reported unanimously a gradual decrease in renal

blood flow beginning on 3 rd to 4 th day or a precipitous fall in the blood flow on later days after the transplantation. On the contrary, the result from present experiments exhibited the abrupt and steep decrement in the renal cortical blood flow occuring at some time from 3 rd to 5 th postoperative day. During the course of these experiments, a report by RETIK and his coworkers[37] appeared showing an early progressive decrease in cortical blood flow by the measurements of total renal blood flow and distribution of intra-renal blood flow using an electromagnetic blood flow meter and inert gas washout technique. The result of their investigation is quite similar to that of this experiment.

It is decisively confirmed that the steep circulatory decrement occurs in the early rejection period in the cortical tissue of the canine allografted kidney. Nevertheless, the cause of the sudden decrease of cortical blood flow in the renal allograft still remains obscure. It is considered that the ischemia in renal allograft rejection must result not only from the irreversible damages, such as the dissolution and blockage of small intertubular blood vessels, but also from the reversible changes, such as interstitial edema and vascular spasm ; and the latter would occur earlier and might be conducive to the progression of the former because the reversal of the established allograft rejection was noted by many investigators.

The role of interstitial edema and swelling of arteriolar endothelium in the ischemia of renal allograft rejection has not been clarified. DEMPSTER[10] is of the opinion that edema does not cause the anuria as he observed that an allografted kidney increased threefold in size and weight within 72 hours and continued in that state for several days afterwards. On the other hand, KOUNTZ et al.[28] considered that the edema was one of the cause of the reduction of total renal blood flow. By the measurement of the wet weight/dry weight ratios, WILLIAMS et al.[54] suggest that the process of homotransplantation incurs a certain amount of edema, and oliguric allografts are consistently more edematous than functioning kidneys. The steep decrement in cortical blood flow followed by progressive swelling of transplants was observed in the canine renal allografts in the experiments presented here. It would seem probable that the vasospasm brings about edema, and some vicious circle intervenes between vasospasm plus edema and ischemia in the kidney allograft rejection ; then the renal allograft suddenly becomes anuric.

It is thought that further information on the mechanism of the sudden blood flow decrement might be obtained with analysis of the effects of vasoactive and immunosuppressive drugs upon the renal allograft rejection. Cortison has been employed by many workers in an attempt to alleviate the immunologic reaction ; but the descriptions of the effect of this drug on the modification of allografted kidneys are mutually inconsistent as are those on the survival of allografted skin. Cortisone has been found by some investigators to prolong the survival of skin allograft[4)5)7)], and by others to have no effect in this regard[13)51)52)]. Some investigators reported that cortisone did not prolong the survival of renal allografts in the dog and human beings[33)32)20)]. HOLLENBERG et al.[18] measured the renal blood flow with an electromagnetic flow meter and reported that the intra-arterial administration of corticosteroids were not capable of reversing the ischemia in the canine renal allograft. On the other hand, it was reported by several workers that cortisone prolonged the renal allograft survival and reversed the incipient rejection in the dog

and human beings[5,6,11,31,45,34]. KOUNTZ et al.[29], using ^{131}I Hippuran clearance, reported the evidence suggesting an increase in renal blood flow by the administration of methylprednisolone succinate. It was also observed in the experiment described here that the gradual recovery of blood flow in the canine renal allograft undergoing rejection resulted with the bolus administration of prednisolone starting about 15 minutes after the injection of it.

Despite of this large amount of literature, the mode of action of cortisone on the kidney allograft rejection has not been understood. One explanation may be that cortisone can not suppress the essential immunologic reactions, such as plasma cell infiltration, but can repair the additional reactions, such as edema and vasoconstriction. By the arteriograms, India ink injection, and direct measurement of the venous outflow, DEMPSTER[10] found that the blood flow in cortisone-treated allografts was usually better maintained than that in the untreated allografts. And he postulates that the irreversible vascular spasm, due to a severe antigen-antibody reaction, is the prime cause of the arrest of function of the allografted kidney. Therefore, it seemed to be indicated to elucidate whether the vascular tone increase does occur in the renal allograft undergoing rejection or not.

Many structures have been shown to develop a hypersensitivity to epinephrine following denervation and it has been shown that the denervated kidney also exhibits this response to epinephrine[17,30]. BERNE et al.[3] reported that the chronically denervated kidney exhibited a greater sensitivity than normal innervated kidney to nor-epinephrine, and that this increased sensitivity was present 9 days after denervation and persisted unchanged for 45 days. In the present experiments, the hypersensitivity to nor-epinephrine was also shown in the cortical vascular system of auto- and allografted kidneys. Since the renal allograft is completely denervated, if some vasoconstrictive agents are produced with immunologic reactions and affect the allografted kidney, vascular tone will be liable to increase. And, if the vascular tone has already increased in the renal allograft undergoing rejection, the response of vascular system to nor-epinephrine in the allograft might be evoked less than that in the autograft. In the current experiments, however, the response of the renal allograft to nor-epinephrine was quite similar to that of the autograft, so that the vascular tone increment derived from the immunologic reactions could not be demonstrated in the allografted kidney.

Information of the vascular tone caused by rejection might also be obtained by the study of the vascular sensitivity of renal allograft to vasodilator drugs. HOLLENBERG et al.[19] reported a marked increase in vascular sensitivity in the kidney allograft to acetylcholine not found in the autograft. In the present experiments, the slight increase in cortical blood flow was observed in the renal allograft undergoing rejection with the intravenous administration of acetylcholine. However, it would be erroneous to compare the current result directly with that of HOLLENBERG since the mode of drug administration in these experiments is quite differ from that of HOLLENBERG. It is suspected that the vascular response of the kidney allograft to acetylcholine might be shaded by the reduction of blood pressure induced by the same drug.

For the satisfactory solution of these problems, further investigation with more improved methods is required. The method of blood flow measurement reported here is

not entirely free from technical difficulties. The fineness of the wires, although seemingly desirable from the standpoint of the accuracy of flow measurement, may well have been a factor in the difficulties of successive flow measurement. The thermocouples and the leading wires were sometimes damaged by the dogs. And the thermocouple which was successfully preserved could rarely be used again because the leading wires had densely adhered to the surface of the kidney at the termination of the experiments, and were difficult to pull out from the kidney without some troubles. Each thermocouple has a differ character in its generation of electric power. In the present experiments, the semi-quanti ative measurement was made on the cortical blood flow because the calibration of electric character could not be made on all double thermocouples.

In the laboratory of this surgical division, SOMA and TSUNEKAWA, and their co-workers[50] are constructing and examining the advanced thermo-electric flow meter and the improved electric circuit with feed back loop to control the heating current. It is hoped that the investigation using this new device will provide more precise information than that obtained in the experiments described here. Moreover, the improved thermo-electr'c flow meter can be put to clinical use, and more satisfactory results will be obtained with this method in the human renal transplantation in the near future.

SUMMARY

The serial and continuous measurements of the cortical blood flow were performed using double thermocouple embedded in the cortical tissue of normal, autografted, and allografted kidneys in the dogs. The results from these experiments have assisted the author in reaching the following conclusions.

1) The experiments reported here proved the feasibility of serial and continuous studies on the hemodynamics in the renal cortical tissue with double thermocouple method.
2) It is decisively confirmed that the abrupt and steep blood flow decrement occurs in the cortical tissue of renal allograft in the early rejection period. This phenomenon appears 1 to 3 days earlier than other indices of rejection crisis, and therefore the earlier detection of rejection crisis can be made with this method.
3) The effect of immunosuppressive treatment can be monitored with this method in the allografted kidney. It is ascertained that the renal allograft rejection in the early stage can be reversed with the administration of adrenocortical steroids ; and the response of blood flow to the therapy is brought about in the cortical tissue within 4 hours following the administration.
4) It is also clarified that the transplanted kidney which loses control of nervous system gradually acquires a hypersensitivity to vasoconstrictive agents ; the sensitivity of the cortical tissue to nor-epinephrine shows a 100 % increase in the transplanted kidney over the normal kidney by the 10 th postoperative day. It is considered that the transplanted kidney might be brought under the control of the humoral system by this phenomenon within 10 days following the transplantation.

The response of the renal allograft to adrenocortical steroids, nor-epinephrine, and acetylcholine was discussed in relation to the edema and the vasoconstriction which was the possible cause of abrupt and steep circulatory decrement of the cortical tissue in the

renal allograft rejection in dogs.

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REFERENCES

1) Baker, R., Gordon, R., Huffer, J. and Miller, G. H., Jr. Experimental renal transplantation. I. Effect of nitrogen mustard, cortisone and splenectomy. Arch. Surg., **65** 702, 1952.
2) Baldes, E. J. and Herrick, J. F. : A thermostromuhr with direct current heater. Proc. Soc. exp. Biol. Med., **7** 432, 1937.
3) Berne, R. M., Hoffman, W. K., Jr., Kagan, A. and Levy, M. N. : Response of the normal and denervated kidney to l'epinephrine and l'nor-epinephrine. Amer. J. Physiol., **171** 564, 1952.
4) Billingham, R. E., Krohn, P. L. and Medawar, P. B. : Effect of cortisone on survival of skin homografts in rabbits. Brit. Med. J., **1** : 1157, 1951.
5) Billingham, R. E., Krohn, P. L. and Medawar, P. B. : Effect of locally applied cortisone acetate on survival of skin homografts in rabbits. Brit. Med. J., **2** : 1049, 1951.
6) de Burgh Daly, I. and Verney, E. B. : Cardiovascular reflexes. J. Physiol., **61** : 268, 1926.
7) Cannon, J. A and Longmire, W. P., Jr. : Studies of successful skin homografts in the chicken. Ann. Surg., **135** : 60, 1952.
8) Darmady, E M., Dempster, W. J. and Stranack, F. The evolution of interstitial and tubular changes in homotransplanted kidneys. J. Path. Bact., **70** 225, 1955.
9) Dempster, W. J. Kidney homotransplantation. Brit. J. Surg., **40** 447, 1953.
10) Dempster, W. J. The effects of cortisone on the homotransplanted kidney. Arch. internat. pharmacodyn., **95** 253, 1953.
11) Dempster, W. J. : A Consideration of the cause of functional arrest of homotransplanted kidneys. Brit. J. Urol., **27** : 66, 1955.
12) Ellison, E. H., Martin, B. C., Williams, R. D., Clatworthy, H. W., Hamwi, G. and Zollinger, R. M. : The effect of ACTH and cortisone on the survival of homologous skin grafts. Ann. Surg., **134** : 495, 1951.
13) Franklin, D. L., Baker, D. W. and Rushmer, R. F. : Pulsed ultrasonic transit time flowmeter. I. R. E. Trans. Bio-Med. Electron., **9** 199, 1962.
14) Gibbs, F. A. A thermoelectric blood flow recorder in the form of a needle. Proc. Soc. exp. Biol. Med., **31** : 141, 1933.
15) Grayson, J. : Internal calorimetry in the determination of thermal conductivity and blood flow. J. Physiol., **118** 54, 1952.
16) Gregg, D. E., Pritchard, W. H., Eckstein, R. W., Shipley, R. E., Rotta, A., Dingle, J., Steege, T. W. and Wearn, J. T. : Observations on the accuracy of the thermostromuhr. Amer. J. Physiol., **136** : 250, 1942.
17) Hartmann, H., Orskov, S. L. and Rein, H. : Die Gefaßreaktionen der Niere im Verlaufe allgemeiner Kreislauf-Regulationsvorgänge. Pflügers Arch. ges. Physiol., **238** 239, 1936.
18) Hollenberg, N. K., Retik, A. B., Rosen, S. M., Murray, J. E. and Merrill, J. P. : The role of vasoconstriction in the ischemia of renal allograft rejection. Transplantation, **6** : 59, 1968.
19) Horowitz, R. E., Burrows, L., Paronetto, F. and Wildstein, W. : Immunocytochemical observations on

canine kidney homografts. Fed. Proc., **22** : 274, 1962.

20) Hume, D, M., Merrill, J. P., Miller, B. F. and Thorn, G. W. : Experiences with renal homotransplantation in the human : report of nine cases. J. clin. Invest., **34** 327, 1955.

21) Jackson, B. T. and Mannick, J. A. Serial blood flow in first set renal homotransplants undergoing rejection. S. G. O., **119** : 1265, 1964.

22) Janigan, D. T., Williams, M. A., Tyler, H. M. and Dempster, W. J. : A biochemical approach to the study of rejection of canine renal homotransplants. III. Histochemical studies. Brit. J. exp. Path., **45** 347, 1964.

23) Katsura, S., Weiss, R., Baker, D. and Rushmer, R. F. Isothermal blood flow velocity probe. I. R. E. Trans. Med. Electron., **6** : 283, 1959.

24) Kety, S. S. and Schmidt, C. F. . The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. Amer. J. Physiol., **143** : 53, 1945.

25) Kiese, M. and Lange, G. Calorimetrische Messung der Durchblutung des Herzmuskels. Arch. exp. Path. Pharmakol., **231** : 149, 1957.

26) Knudsen, D.F., Davidson, A. J., Kountz, S. L. and Cohn, R. . Serial angiography in canine renal allografts. Transplantation, **5** : 256, 1967.

27) Kolin, A. : Electromagnetic blood flow meters. Science, **130** : 1088, 1959.

28) Kountz, S. L., Williams, M. A., Williams, P. L., Kapros, C. and Dempster, W. J. : Mechanism of rejection of homotransplanted kidneys. Nature, **199** 257, 1963.

29) Kountz, S. L., Laub, D. R. and Cohn, R. Detecting and treating early renal homotransplant rejection. J.A. M.A., **191** 997, 1965.

30) Kubicek, W. G., Harvey, R. B. and Kottke, F. J. : The adrenalin sensitivity of the denervated dog kidney. Fed. Proc., **7** : 68, 1948.

31) Marchioro, T. L., Axtell, H. K., Lavia, M. F, Waddell, W. R. and Starzl, T. E. The role of adrenocortical steroids in reversing established homograft rejection. Surgery, **55** 412, 1964.

32) Murray, J. E., Merrill, J. P., Dammin, G. J., Dealy, J. B., Jr., Alexandre, G. W. and Harrison, J. H. Kindney transplantation in modified recipients. Ann. Surg., **156** 337, 1962.

33) Persky, L. and Jacob, S. Effect of ACTH and cortisone on honogenous kidney transplants. Proc. Soc. exp. Biol. Med., **77** 66, 1951.

34) Porter, K. A., Thomson, W. B., Owen, K., Kenyon, J. R., Mowbray, J. F. and Peart, W. S. . Obliterative vascular changes in four human kidney homotransplants. Brit. Med. J., **1** : 639, 1963.

35) Porter, K. A., Calne, R. Y. and Zukoski, C. F. Vascular and other changes in 200 canine renal homotransplants treated with immunosuppressive drugs. Laborat. Invest., **13** : 809, 1964.

36) Rein, H. : Die Thermo-Stromuhr. Ztschr. Biol., **87** 394, 1928.

37) Retik, A. B., Hollenberg, N. K., Rosen, S. M., Merrill, J. P. and Murray, J. E. Cortical ischemia in renal allograft rejection. S. G. O., **124** : 989, 1967.

38) Rosen, S. M., Retik, A. B., Hollenberg, N. K., Merrill, J. P. and Murray, J. E. Effect of immunosuppressive therapy on the intrarenal distribution of blood flow in dog renal allograft rejection. Surg. Forum, **17** : 233, 1966.

39) Rosen, S. M., Truniger, B., Kriek, H. R., Oken, D. E., Murray, J. E. and Merrill, J. P. : Intrarenal distribution of blood flow in normal and autotransplanted dog kidneys. Effect of hemorrhagic hypotension and mannitol. J. clin. Invest., **44** 1092, 1965.

40) Schmidt, C. F.and Walker, A. M. A thermostromuhr operating on storage-battery current. Proc. Soc. exp. Biol. Med., **33** : 346, 1935.

41) Shillingford, J., Bruce, T. and Gabe, I. : The measurement of segmental venous flow by an indicator dilution method. Brit. Heart J., **24** : 157, 1962,

42) Shipley, R. E., Gregg, D. E. and Wearn, J. T. Operative mechanism of some errors in the application of the thermostromuhr method to the measurement of blood flow. Amer. J. Physiol.,**136** : 263, 1942.

43) Simonsen, M., Buemann. J., Gammeltoft, A., Jensen, F. and Jorgensen, K. : Biological incompatibility in kindney transplantation in dogs. I. Experimental and morphological investigations. Acta path. bicrob. Scand., **32**: 1. 1953.

44) Soskin, S., Priest, W. S. and Schultz, W. J. : The influence of epinephrine upon the exchange of sugar between blood and muscle. Amer. J. Physiol., **108**, : 107, 1934.

45) Starzl, T. E., Marchioro, T. L. and Waddell, W. R. : The reversal of rejection in human renal homografts

with subsequent development of homograft tolerance. S. G. O., **117** : 385, 1963.
46) 高橋 功：交叉熱電対法による肝循環動態の薬理学的研究，日薬理誌，**60** 308, 昭39.
47) Tigerstedt, R. Studien über die Blutvertheilung im Korper. Skandinav. Arch. Physiol., **3** 145, 1892.
48) Truniger, B., Stanley, M., Rosen, M. B., Kriek, H., Merrill. J. P. and Murray, J. E. Intrarenal distribution of blood flow in the rejecting homotransplanted dog kidney. Surg. Forum, **16** 254, 1965.
49) 恒川謙吾・町塚 昭・南 亮・池田正尚・大串直太・熊田 馨：疼痛に伴なう一，二の問題．最新医学，**23** : 756, 昭43.
50) Tsunekawa, K., Mohri, K., Ikeda, M., Ohgushi, N., Soma, T. and Sawai, T. Measurement of blood flow by heated thermocouple with feedback controlled current. Experientir, **24** 1077, 1968.
51) Weisman, P. A., Quinby, W. C., Wight, A. and Cannon, B : The adrenal cortical hormones and homografting : exploration of a concept. Ann. Surg., **134** 506, 1951.
52) Weisman, P. A., Wight, A., Quinby, W. C. and Cannon, B. . The failure of adrenal cortical hormones to prolong the survival of homologous skin grafts. Plast. Reconstruct. Surg., **8** : 417, 1951.
53) Williams, M. A., Tyler, H. M., Morton, M., Nemeth, A. and Dempster, W. J. Some biochemical changes in the transplanted kidney. Brit. Med. J., **2** : 1215, 1962.
54) Williams, M. A., Morton, M., Tyler, H. M. and Dempster, W J. . A biochemical approach to the study of rejection of canine renal homotransplants. II. Chemical analysis of kidney homogenates. Brit. J. exp. Path., **45** : 235, 1964.
55) Williams, P. L., Williams, M. A., Kountz, S. L. and Dempster, W. J. : Ultrastructural and haemodynamic studies in canine renal transplants. J. Anat. Lond., **98** : 545, 1964.
56) Zukoski, C. F., Callaway, J. M. and Rhea, W. G., Jr. : Prolonged acceptance of a canine renal allograft achieved with prednisolone. Transplantation, **3** 380, 1965.

和文抄録

犬同種移植腎に於ける拒絶反応発現時の皮質内血流動態の経時的観察

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自家及び同種移植腎皮質内の血流動態の変化の状態を，経時的及び持続的に検討し，以つて人同種移植腎の拒絶反応の把握に資せんがため，双熱電対による血流測定法を用いて，以下の実験を実施した．

1) 正常腎にこの方法を適用して，同法の安定性と腎に対する影響を検討した．

2) 自家移植腎に於いて，皮質内血流の経時的観察により，腎移植術の影響を調査した．又Nor-epinephrineを用いて，腎移植に伴う神経切断の血流に及ぼす影響を持続的観察により検した．

3) 無処置の同種移植腎に於いて，拒絶反応支配下の腎皮質内血流の変化を経時的に観察し，単純X線像により得られた腎腫脹の進行，血管造影像，及び組織学的検索の結果と比較検討した．

又，静注によつて投与したPrednisolone. Nor-epinephrine及びAcetylcholineに対する，同種移植腎の皮質内血流の反応を持続的測定により観察した．

4) Azathioprineによつて処置した同種移植腎の血流変化を経時的に観察した．又同時にPrednisoloneの投与を加えて，血流に及ぼす影響を経時的に観察した．

以上の実験より，下記の結果と結論を得た．

1) 熱電対及び導線の断線により，実験の中止をしばしば余儀なくされたが，本法による腎皮質内血流の経時的観察の可能性が明らかとなり，これによつて腎に認むべき障害を加えぬ事も判明した．

2) 自家移植腎に於いて，術中の出血による影響の他には，皮質内血流の著るしい変動を認めなかつた．又皮質内血管系のNor-epinephrineに対する感受性が，移植術後数日中に次第に増強する事実が明らかになつた．又，血流のNor-epinephrineに対する反応曲線が，単峰状から2峰状に変化する模様が観察された．

3) 同種移植腎について，腎腫大，尿量減少等の明らかな拒絶反応の出現に1～3日先行し，第3～5手術日に皮質内血流の急激な減少の起る事を観察した．この際Prednisoloneを静注し，約15分後より持続的且緩徐な血流量の増加が始まる事を認め，Acetylcholineの静注により，直後より約3分間続く僅かな血流増加を認める事があり，Nor-epinephrineに対する反応が自家移植腎のそれと全く同様である事を観察した．

4) 同種移植腎に於いて，Azathioprineにより拒絶反応を抑制する時，皮質内血流も良く保たれ，Azathioprine減量により，血流も減少してゆく事，及びこの際Prednisoloneの投与により約4時間以内に血流の部分的回復が完了する状況を観察した．

以上の結果より，次の如き推論を行ない，且これに検討を加えた．

1) 移植により神経支配より完全に離脱した腎は，数日中に血管収縮性物質に対する過敏性を獲得し，数日中に体液性支配を受けるに至る事が考えられる．

2) 移植腎皮質内血流のNor-epinephrineに対する反応曲線が2峰性に変化する事は，腎皮質内α-Receptorの作用の複雑性を物語るものか，或はα-Receptorが複数の要素より構成されている事を暗示するものである．

3) 腎同種移植時の拒絶反応の細部は殆んど未知のまま残されているが，現象面の主体をなすものがIschemiaである事はすでに多くの推論がなされ，本実験により，それが皮質内のIschemiaである事が確証された．更にこの原因をなすものは恐らくはVasospasmであると考えられ，Edemaがこれに伴ない，Ischemiaをもたらすものと思われる．これを暗示する事実は多いか，本実験においては，拒絶反応の初発時に，即ち皮質内血流が減少をはじめた時点で，Vasospasmを立証する事に失敗した．今後実験方法の改善と，測定器の改良により事実の解明される事を期待している．